Substance Abuse and Mental Health Services Administration

Drug Testing Meeting Board (DTAB)

Open Session

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P R O C E E D I N G S (9:30 a.m.)

Agenda Item: Call to Order, Brian Makela, Designated Federal Officer

MR. MAKELA: Good morning, everyone, and welcome to the September quarterly meeting of the Center for Substance Abuse Prevention's Drug Testing Advisory Board. My name is Brian Makela, and I am the designated federal officer. I officially call this open session of the DTAB to order. Today's open session is scheduled to start at 9:30 a.m. and ends at 12:45 p.m.

I'd like to welcome our board members, the Division of Workplace Programs staff, our federal partners from the Department of Transportation, Department of Defense, Office of General Counsel, Nuclear Regulatory Commission, and the Office of National Drug Control Policy. I would also like to welcome contractors, members of the public, and invited guests.

This morning, portion of today's meeting is an open session with presentations from Division of Workplace Programs staff and also from personnel at RTI International. Topics include program updates, results from the opioid implementation performance testing samples, lessons learned from the oral fluid pilot PT program, stability of DNA in specimen testing matrices, and detection of opioid metabolites in user hair.

SAMHSA invited the public to register for comments at this meeting and no one registered to make public comments at this time.

This afternoon's session and the session tomorrow are closed sessions where we will discuss proposed revisions of the mandatory guidelines, hair practices and procedures, and future planning of board activities. The closed session is only available to board members and invited guests.

Today's presentations are available through Adobe Connect. The web conference address was provided with meeting registration. For those who called in to the teleconference, you will be in listen-only mode. For our presenters, please make sure to mute any devices and minimize background noises during your presentations. For all those who are speaking, please identify yourselves clearly for the transcriber.

As you can see here, these are the proposed dates for the four quarterly meetings of the Drug Testing Advisory Board in fiscal year 2018. Two of these meetings will be one day and take place via web and teleconference attendance only, and two meetings are scheduled to take place onsite at SAMHSA headquarters over the course of two days with the option to attend by web conference.

The topics to be presented at these are yet to be determined and it has not been determined if these meetings will be opened or closed or a mixture of the two. A notice with the description of topics and the open/closed nature of the meeting will be published in the Federal Register approximately two weeks prior to each meeting date. You can find public information about the board, its past meetings, presentations, transcripts, and meeting summaries at the website listing here. Information from this meeting will be available in approximately three to four weeks.

Thank you again to everyone for attending. I would now like to introduce the director of the Division Workplace Programs as well as the chairman of the Drug Testing Advisory Board, Mr. Ron Flegel. He will get us started with introductory remarks and a quick update on advisory board and program activities.

Agenda Item: Welcome and Introductory Remarks, Ron R. Flegel, BS, MT (ASCP), MS, Director, Division of Workplace Programs, CSAP, SAMHSA

Agenda Item: Mandatory Guidelines for Federal Workplace Drug Testing Programs – Update, Ron R. Flegel

MR. FLEGEL: Thank you, Brian. I would also like to thank the board members, ex officios, industry representatives, and members of the public for taking time

out of their schedules today to be with us in the Drug Testing Advisory Board meeting.

Over the next several minutes, I'll have a presentation where I will update you on the progress of the mandatory guidelines for both urine and oral fluid and the progress of the mandatory guidelines for hair, some of the Division of Workplace Programs initiatives we are working and other programmatic information that the HHS-certified laboratories, federal agencies, and drug testing industry as well as the public may find helpful.

As Brian mentioned, we do have a number of presentations today including the opioid implementation proficiency testing data, a pre-study in the stability of DNA in the urine and oral fluid, and some information on the data on opioid metabolites in hair.

I would also like to say that I would like to introduce the new assistant secretary if she is able to join us today. It's Dr. Elinore McCants-Katz and she was confirmed about a month ago. So if she comes in due to her schedule, I will introduce her. I'm sure she would like to say a few words with the board.

SAMHSA seeks to improve the quality of services for forensic workplace drug testing, assess the science and technology used in the drug analysis, improve the quality of related laboratory services, and systems of drug

testing, and to also formulate standards for laboratory certification for federal workplace drug testing programs and guide national policy in these areas with the Office of National Drug Control Policy.

The CSAP DTAB provides advice, again, to the Assistant Secretary for Mental Health and Substance Use based on the ongoing review of the direction, scope, balance, and emphasis of the agency's drug testing activities and the drug testing laboratory certification program. With that, I will also say that we are looking to update the charter. One of the things that we do need to update the charter in is around the assistant secretary where it actually states the administrator right now.

With that, I will go ahead and get started on the presentation. We did have the National Laboratory Certification Program workshop on Friday; although SOFT was postponed, we did want to go ahead and have that and inform the laboratories of all the information around the NLCP. So I think that went over well.

There weren't as many questions as we thought there would be. So again, as I go through this presentation, if the board members do have questions, feel free to stop me, ask those questions at that time.

Again, this is just welcoming and opening remarks. Again, the staff at the Division of Workplace

Programs, we are down one person, Jennifer Fan, who was our pharmacist. She is now in CSAP. She's moved away from the Division of Workplace Programs, but unfortunately, we do not have a position open that we can fill right now.

Again, regulation and policy, there's a number of things we look at starting with the donor, the drug test, the medical review officers, all the way down to the Drug Testing Advisory Board. With all the changes and all the updated information we've had to do implementing the urine revised guidelines, we've had to go through a number of these things and look at all this information that we put out. So again, I just wanted to touch on this regulation and policy part.

The Drug Free Workplace Program, as a federal entity, we work with a number of federal laws, the testing issues, specifically right now when it comes to state legislation specifically around marijuana, state laws, and then contract and legal issues. As we revised the mandatory guidelines, there was a number of those things that came in when it came to both contracts as well as legal issues.

DWP objectives and goals, at present is the implementation of the revised urine mandatory guidelines. We're in the process of approving oral fluid as an alternate specimen in the federal workplace drug testing

program. Future would be to write the proposed hair mandatory guidelines. The overall goal right now we're looking at is implementing the semisynthetic opioid testing in the regulated programs, specifically around hydrocodone, oxycodone, hydromorphone, and oxymorphone. A big task, a big thing there. Again, with the opioid crisis that everyone has heard around in the United States, it's very important to the secretary as far as the implementation of testing for the semisynthetic opioids.

Federal Register notice was published January 23, 2017 with an implementation date of October 1, 2017. The changes just in general were obviously the added semisynthetic opioids. We removed MDEA. There was not a number of significant positives there when we looked at that, though we did add MDA as an initial screening analyte. We raised the lower pH cutoff, for adulterated specimens, from 3 to 4. That also changed the invalid range, made it smaller, from 4 to 4.5.

But this, again, when we did a lot of studies within NLCP, we looked at this specifically because we felt there was a lot of, whether they were synthetic urines or substitute samples that were coming into the laboratories that met the pH criteria, but were obviously after testing, they were not regular routine urine samples. They were something, whether they were synthetic or they could have

been apple juice. There are a number of other issues with that. So we don't know the percentage increase we'll see in adulterated, but I'm sure there will be a percentage increase within the laboratories.

Then there were many wording changes to address the alternate specimens, specifically oral fluid, when authorized within the urine mandatory guidelines. I do want to reiterate that the oral fluid mandatory guidelines are not final. So where we do refer in the revised urine mandatory guidelines, again, an MRO does not have the ability to order an alternate matrix being oral fluid at this time until we have a final published notice. That was a question that did come up so I wanted to add that.

Within the revised mandatory guidelines effective date, the HHS-certified laboratories are on target to meet the October 2017 effective date and again, we will show some of the qualifying performance testing samples, the data here. Also, HHS does understand there were many laboratory information or IT issues around this change with the nonuniform implementation, but again, we are here to help in any way that we can when it comes to that and answer questions if we can.

The federal agency drug program coordinators have been sent and it will be called an assurance letter, or its statement that the Agencies Drug Free Workplace Programs

have been changed to be consistent with the requirements in the mandatory guidelines. That is, one of the things is around the testing of the semisynthetic opioids.

Again, reiterate again, HHS Secretary's priority is on the opioid crisis. There are a number of initiatives moving forward within the federal government on the opioid crisis. And again, the testing for the semisynthetic opioids could help to provide treatment for employees in federal agencies and deter the illicit drug use of prescription opioids. That is hopefully one of the benefits of implementing the revised guidelines.

The new CCF will be in effect for federal agencies. The previous 2014 CCF has been extended to June 1, 2018. So the new CCF will have the semisynthetic opioids on that. I don't have a presentation. Charlie did that presentation last DTAB meeting. So again, it is out there, it's been downloaded, it's been sent to the laboratories through the National Laboratories Certification Program. But again, the old CCF has been extended until June of 2018. The new CCF will be implemented on October 1, 2017.

Around oral fluid mandatory guidelines, with the THC/THCA issue, there are technical and scientific peer reviewed journal articles. We've done a number of those now. Again, NLCP provided a list of those references to

the directors, MROs, inspectors. I don't think that's been sent out. Again, we can send that out to the board, members of the public. What we're hoping to do is get this published on our website so that people can see the scientific and peer reviewed journal articles.

Again, I want to thank Dr. Ed Cone, who's here today, and Dr. Ryan Vandrey from Johns Hopkins University for doing those studies. It's been a tremendous thing, not only within our sector of the drug testing, but also in other parts of programs, whether it's DUID or other parts of programs that use oral fluid.

I just put a proposed implementation date. Again, this is questionable, but 2018 is what we're looking at. I know that is fast approaching when it comes to oral fluid mandatory guidelines.

The inclusion of testing oral fluid at the matrix in the federal program I think is going to be important. Again, we don't have an alternate specimen we can use at the present time. Again, when oral fluid is implemented, we will have an alternative specimen.

Again, the one thing around semisynthetic opioids for oral fluid is they have obviously been added to the federally regulated drug testing panel under the proposed oral fluid mandatory guidelines, but again I think it's important, not necessarily to the drug testing but also to

the impairment issue when it comes to oral fluid, what you're looking at as far as parent drug or metabolite. I know in law enforcement, et cetera, this is an informative thing when you're looking at the impairment issues around oral fluid equating with blood. I think some of the data that we've shown in the science around oral fluid shows that there is a good correlation between oral fluid and blood levels.

The new mandatory guidelines for oral fluid, HHS continues to look at options for which marijuana analytes may be used. There doesn't seem to be any single immunoassay to detect both THC and THCA at the proposed oral fluid cutoffs. There is one commercial THCA assay, but it does have significant cross-reactivity to THC.

Then laboratories, from the proposed oral fluid mandatory guidelines, laboratories do have the ability to use an alternate method other than an immunoassay. That also goes for urine. But again, I just wanted to point this out that within oral fluid, there is a now alternate method that you can use other than immunoassays. The testing for parent drug, as I mentioned, the psychoactive component of cannabis, the THC, is very important for the use under Driving Under the Influence of Drugs. Then HHS, just to reiterate under that, does not accept passive exposure as a reason to test positive.

Just an update on the hair mandatory guidelines, DWP staff is currently writing the draft proposed hair mandatory guidelines. There are some proposed research studies around the unique metabolites that we saw last time that DTAB was presented. We do have another presentation today on some of those.

The Secretary's approval of the Drug Testing Board, and again this was in 2015 or 2014, around the Drug Testing Advisory Board's recommendation to pursue hair as an alternate specimen, with two caveats to that. I'll get to that. Then the scientific and technical issues have to be addressed before we can really finish the proposed mandatory guidelines for hair.

DTAB's recommendation was to pursue hair testing, just to reiterate. There were two issues around that. One was the decontamination of hair specimens and also the hair color impact. Again, SAMHSA continues to develop the proposed hair mandatory guidelines.

Some of the ongoing challenges we have will be implementing the oral fluid mandatory guidelines in a program. This program has always used urine since 1988. So that will be a challenge, I feel. The funding of this new program, the review of the technical and scientific study to support the hair decontamination procedures or unique biomarkers or metabolites to rule out external

contamination, and then addressing DTAB's recommendation overall, and then also, as we're all aware, not only around the emerging issues when it comes to opioids, but also synthetic drugs in general, whether it's marijuana, synthetic marijuana, synthetic drugs, and also the legislation and state laws that seem to be changing daily.

Some of the opportunities are implementing the revised mandatory guidelines for urine including the semisynthetic opioids, the oversight and standardization of the regulated industry in the testing for the semisynthetic opioids, and again, we hope that this deters the illegal use of drugs and prescription opioids. I have just a small study I was going to show just for information.

Then opportunity to implement oral fluid as an alternate specimen, therefore decreasing the number of substituted and adulterated specimens. As Dr. Barry Sample showed last time with the Quest data around oral fluid as compared to urine, there seems to be a significant positivity rate in oral fluid over urine. Some of that we feel is due to these adulteration products. Then it also does allow federal agencies a noninvasive alternative to urine testing.

This slide I've showed for the routing process. It can extend from one month to 15 years I think sometimes. So we are out of this routing process for urine. We have

completed that. That is now in the implementation phase. I believe from this slide, if you can see the number, with the oral fluid we're at 15 and with hair we're modestly in front of the process. I know some people do follow this closely.

The MRO guidance manual is out. We sent it out through the NLCP to laboratories, MROs, et cetera, last week. We are going to post it on our website. We hope within the next few days that will be posted on the website. We are having a couple issues around the Adobe fillable information which has to be 508-compliant. So we're working on that. We're trying to get that posted.

Again, if people haven't received that, please let me know because we're trying to get it out to everyone so they have the ability to review this, obviously before the October 1 date. Sean Belouin, if there are any questions, you can refer it to SAMHSA or Sean specifically for that.

Again, I think the addressing of the addition of the prescription opioid drugs is very important. Again, just to reiterate, the hydrocodone combination drugs were moved from a schedule III to a schedule II about a year and a half ago, which is important within our realm of what we do because we can test for both schedule I and schedule II.

Then the biggest question I think surrounds everything is, what is considered a valid prescription under the Drug Free Workplace Program and how will it be interpreted by the medical review officer? Hopefully within the MRO manual, we've given a lot of guidance around that. I want to also say that within the studies that we've done around the single dose opioid, actually, for all the semisynthetic, there are very good curves and graphs in there that I think MROs should be aware of. A lot of this information in the references are also referenced in the MRO manual, which is important.

Then in subpart M, under the medical review officer, section 13.1 is where a lot of this information is found.

Some of the ongoing studies, out of the other ones that we've completed, we also wanted to do a cannabidiol study, which unfortunately has been postponed. We wanted a start date in August 2017 but because of some approvals in that, we had to push that back to the next fiscal year. Again, around that study, there is a lot of CBD oils that you can buy on the internet and have it shipped. They aren't supposed to be shipped, but they are shipped. With that, we've had a number of calls where individuals have said I'm taking this for medicinal purposes. I tested positive in my Drug Free Workplace

Program; why is that? They said you can't test positive. So this study I think is important. We've looked at what, when it comes to cannabidiol preparations around oils, how much percentage of THC is within that.

Two presentations that were going to be given at SOFT were the disposition of cannabinoids in oral fluids and whole blood after vaporized and smoked cannabis. We hope in the future we'll be able to give that here. I think that's very important now, that we can tie all three of those together, both the passive exposure, the ingestion with edibles, and also the vaporized. I think it's really good to see all those put together.

Then the other presentation at SOFT, which will, again, now be in January, are the pharmacodynamic comparison of acute cannabis effects following oral, smoked, and vaporized administration.

This was the study I was talking about. This is a retrospective study of samples that one of the laboratories did for us just to look at, the opioids under both the current guidelines as well as the revised guidelines. So under the current guidelines, we had one confirmed positive out of 520 samples. That was for morphine. Under the revised guidelines, when it was tested, we had nine. We had 1 morphine, 5 hydrocodone, 5 hydromorphone, 1 hydromorphone, 1 oxycodone/oxymorphone,

and 1 oxymorphone. So again, that's a tenfold increase just to be aware.

I know laboratories are looking at that. We've looked at this. We've expected from some of the other studies that we did that the percentage in positivity is going to increase dramatically, at least initially when we start testing.

Marijuana in science, that seems to be the big issue as always, but also with the opioids. Some of the information that I wanted to share, and again I implore you to get these studies that we've completed, the passive inhalation study which has a lot of good data. I think there is a combination of about five different papers on this that we've done. Also, the cannabis brownie study that we've completed, again, we're still writing some additional papers on this around ingestion.

Then also the cannabis vaporization study that's been completed and again, not only with the presentations but also the papers that will be written around that. I think it's amazing to look at some of the data when it comes to that around vaporization.

Through oral fluid, the whole premise of initially why we started this, ONDCP was involved in this, is to also look at oral fluid in federal standards so that within law enforcement and other testing programs, you

could use oral fluid as one of the matrices to test specifically at the roadside. So I just put this slide in there because it was around the oral fluid that we had as far as the standardized cutoff levels. DWP is supporting the methods to minimize the rates of driving under the influence of drugs. If you look at the data and some of the states that have legalized, it's really increasing dramatically, especially over the last year.

DWP has developed guidelines for federal workplace; of course, on drug testing that can be used in other programs including law enforcement.

This is a slide that I wanted to show I guess to sum up all the things. I know it specifically is around drug effect when you look at this, but when you look at ingestion, the timeframe is shifted out pretty dramatically when you look at the actual drug effect or the impairing effect.

But when you look at smoked, it actually is a much lower timeline that you look at about one hour, less than an hour, half hour. So again, I think it's important through all the studies that we've done, when you look at the drug effect, depending on route of administration, where the biggest impact or the high is going to come from whether it's passive inhalation or it's edibles or it's vaporized or it's smoked. There's just a number of things.

Again, the emerging issues are the synthetic drugs I just put here with marijuana, but again it continues to be one of the emerging issues that we come and go, sort of. It actually seems to be increasing a little bit again. Then all the other emerging issues around synthetic drugs in general; specifically there are some other things around synthetic cocaine that is coming out we've read about.

With that, I would like to thank you, and if there are any questions from the board on any of the updates, I will take questions now. Otherwise, I will turn it back over to Brian.

DR. PAUL: This is Buddha Paul. I have two questions. One is that any conflict between the federal and state marijuana, if the state marijuana is legalized, any kind of progress and updates that we can get?

The second question is you were talking about semisynthetic opioids. But that doesn't include fentanyl. Fentanyl is synthetic, not semisynthetic, just to make sure there is a clarification there.

MR. FLEGEL: Again, to go back to the first question, I think there is progress in the sense that FDA kept it a schedule I for marijuana. There has been three legal cases. Two of them have been resolved that I know of within the states that an employer can test for marijuana

even in a state where it's legal and they can uphold that result and fire a person.

The third one I'm not so sure about. That one is still ongoing. Again, some of the premise around that is, like a prescription drug, if you go on your lunch break, let’s says, and you take that prescription drug, does that affect your work when you come back to work and can you test?

So I think there are other issues around that third case. Those are the three I know of. So again, progress, as far as, even, again, not within our program under HHS, but the testing for alcohol, though it's a legal drug, we still test for alcohol. I think that would be the same when it comes to the cannabis or the marijuana.

Around the semisynthetic opioids, if you can just repeat that second part of the question around that. Oh, fentanyl. I was trying to think. We actually are going to do a small study and look at some regulated samples and specifically there were several comments around fentanyl that came in. Should we be testing for it in the program, et cetera? We do obviously test for 6-acetylmorphine and all the opioids, again, semisynthetic.

But again, we wanted to look at a number of regulated samples and see if we could test fentanyl. We're trying to look at right now the assays and who can actually

do that. So we are trying to look at it as far as prevalence if there is a prevalence within the regulated sector.

MS. KELLY: This is Patrice Kelly from the U.S. Department of Transportation. Quick question. Ron, you mentioned that you had the MRO guidelines in circulation and just wanted to clarify -- or circulations for comment. I just wanted to clarify where there is circulation right now.

MR. FLEGEL: Yeah, the MRO manual is actually a guidance document. So we're not really asking for comments. That was one of the concerns, is it's not really going out for comment that would come back in. Again, if there are notable things where there's information, that's essentially that's a show-stopper that we look at, but we would welcome anything that would come back in on the MRO manual regarding those.

MS. KELLY: Patrice Kelly again. Where would somebody who noticed something find it? In other words, where is it if we can't find it on your website?

MR. FLEGEL: Yeah, and again, I think the best thing to do would be contact the Division of Workplace Programs. We'll take that information, what the comment was, and we'll look at the guidelines accordingly or the guidance document accordingly.

We've had a number of reviewers, as has been mentioned earlier in DTAB. Actually, several years ago, is we did have an MRO working group that looked at all of this, looked at all the information and what we wanted to say around the opioids.

Okay, with that, I'll turn it back over to Brian.

MR. MAKELA: Thanks, Ron. Next up I would like to introduce Cynthia Lewallen. She's from RTI International. She's going to talk about the opioid implementation performance testing standards.

Agenda Item: Results from the Opioid Implementation PT Sample, Cynthia Lewallen, MS, Research Forensic Scientist, Center for Forensic Sciences, RTI International

MS. LEWALLEN: Thank you, Brian. So as Brian said, my name is Cynthia Lewallen and I work at RTI International. Just to give you a kind of brief introduction to myself since I don't know most of you, I've been at RTI since 2009. I worked for a couple years in the lab on research projects and then I moved over to working as an inspection analyst for the NLCP. Then within the past year or so, I've been working closely with Dr. Frank Esposito to learn about the design, scoring, and remedial phases of the PT program.

So as you know, all HHS-certified laboratories were required to successfully complete three sets of qualifying PTs to demonstrate their readiness for the changes to the guidelines. So that's what I'm going to give you a summary of today.

On January 23, 2017, the revisions to the mandatory guidelines were published in the Federal Register with an effective date of October 1, 2017. The revisions included the addition of four new analytes, hydrocodone, hydromorphone, oxycodone, and oxymorphone, the addition of MDA as an initial test analyte, and raising the lower pH cutoff from 3.0 to 4.0 for identifying specimens as adulterated. So today I'm going to focus on the addition of the opioids.

On March 7, 2017, the NLCP sent 18 practice samples to all the laboratories. The practice samples were identified with the single analyte present, its target concentration, and the mean concentration from five reference laboratories. The purpose of these samples was to enable the laboratories to verify the performance of their immunoassay and confirmation methods, and the labs were not required to report their results for the practice PT samples to the NLCP.

Qualifying PT sets were shipped approximately six weeks apart, beginning with the first set on May 1 and

ending with the third set on July 24. The first maintenance set of PTs that will incorporate the changes will be shipped on October 9. This set will include an additional 15 samples for a total of 40 PT samples and the labs will be given an additional week for testing. This is a onetime change to the size of the PT set and the time allowed for the laboratories for testing and reporting.

So the new analytes that were added, hydrocodone and hydromorphone, both have an initial test cutoff of 300 nanograms per milliliter. Oxycodone and oxymorphone both have an initial test cutoff of 100 nanograms per milliliter. All four analytes have a confirmatory cutoff of 100 nanograms per milliliter.

Prior to revision of the guidelines, only one laboratory was using LC-MS/MS for their codeine and morphine confirmation tests. The laboratories reported information to the NLCP on the assays they were using for testing the qualifying PTs and that they planned to use after October 1. Eighteen labs will continue using GC-MS and nine labs plan to use LC-MS/MS for codeine and morphine confirmation. Then for analysis of the four added opioids, the use GC-MS and LC-MS/MS are almost equal.

Laboratories were directed to perform initial and confirmatory testing for all analytes on all qualifying PT samples. The samples were designed to challenge the

ability of the laboratories to quantitate over the range of 0.4 to 20 times the confirmatory test cutoff. Samples included potential interferents. Laboratories were directed on a March 15, 2017 notice that their interferent studies for opioids must include norcodeine, norhydrocodone, noroxycodone, noroxymorphone, 6AM codeine, and morphine.

Laboratories were also informed that they must be able to demonstrate the ability to accurately quantify 1,000 nanograms per milliliter of hydromorphone and oxymorphone present as a glucuronide, and samples were included to test the laboratories' hydrolysis efficiency. Samples were also included to test the laboratories' MDA initial test and correct reporting of samples with changes to the invalid and adulterated pH ranges.

This table shows an overview of the distribution of the challenges targeted by qualifying PT samples. So a sample was sent with the analytes at 40 percent of the cutoff and no interferences in each set. This was the only same over all three sets. Samples were sent with the analytes at 40 percent of the cutoff and interferences present in each set. However, the interference and its concentration were different over all three sets.

Set one, the NLCP provided samples with a single analyte from each group of paired analytes with a

concentration at 1.25 times the initial test cutoff to test for the potential for false negatives. So one sample included hydrocodone and oxycodone. So one analyte from the green pair and one analyte from the blue pair. Then the second sample included hydromorphone and oxymorphone. The confirmatory group mean column shows the confirmatory results for those analytes.

Then the initial test kit on here, each column shows the number of labs that obtained a positive initial test using that kit, and the number of labs that used that kit. So using kits that meet the mandatory guidelines' requirements, no false negative results were obtained.

However, I will note here that three labs originally used kits that did not meet program requirements. Either they had cross-reactivity less than 80 percent, they used an analyte other than the analyte specified by the manufacturer as the target, or they grouped together analytes with different initial test cutoffs. Those laboratories were notified that the kits were unacceptable as used and they validated new kits and retested all of the samples from set one.

We provided two samples spiked with glucuronide at approximately four times the initial test cutoff. These samples contained the glucuronide only, no free drug. In the first set, a hydromorphone glucuronide targeted at

1,215 nanograms per mL, or the equivalent of 736 nanograms per mL of free hydromorphone, was sent. All labs used the Thermo Fisher DRI immunoassay for hydromorphone and they all obtained a positive initial test result.

For oxymorphone in the second set, we sent a sample with oxymorphone glucuronide targeted at 403 nanograms per milliliter, or the equivalent of 250 nanograms per milliliter of free oxymorphone. For this sample, all of the labs using the Thermo Fisher DRI and the Lin-Zhi kits contained positive initial test results, but none of the labs using the Siemens EMIT kit obtained a positive initial test result. However, the laboratories were directed to perform both initial and confirmatory tests, and they were only graded on their confirmatory results for these two samples.

The purpose of these samples was for us to challenge the laboratory hydrolysis methods and confirmation, but it also allowed us to gather information on a cross-reactivity of the glucuronides with the immunoassay.

Set two, we sent a sample with each of the opioids at the cutoff. This table shows the mean concentration for each analyte, the plus or minus 20 percent or 2 standard deviation acceptable range and the number of minor errors that were observed. One lab had a

minor error with oxycodone and hydrocodone and one lab had a minor error for all four opioids.

So I'm going to talk a little bit about the errors that we saw and then once I've done that, I'll talk a little bit about the remediation by the laboratories. This table shows the number of minor quantitative errors, that is more than 20 percent but less than 50 percent from the mean for each opioid in each set, then the bottom row I've shown the total number of errors, and then expressed as a percentage of the total number of challenges for that PT set.

Percentages that we saw of errors of minor quantitative errors were similar to the percentages of minor errors that we see for codeine and morphine in maintenance PT sets. As you would expect, or as you would hope, the percentages decrease slightly over each set. I will point out here that for oxycodone and oxymorphone in the third set, there were ten minor errors. Nine out of those ten were made by a single laboratory.

There was only one major quantitative error, that is more than 50 percent from the mean, over all three sets. That was for hydrocodone in a sample that also contained norhydrocodone. For confirmation false negatives, there were 13 confirmation false negatives in set one. Twelve of those 13 were attributed to interference from high levels

of codeine and morphine. Then in set two, we had two false negatives for oxycodone in a sample that contained noroxycodone and noroxymorphone.

In set one, I would like to point out that those 13 errors were not made by 13 different laboratories. Most of those errors were for paired analytes. So if a laboratory had interference that prevented confirmation of oxycodone in the sample, they also failed to confirm oxymorphone in the sample. So that was two of the 13 errors for that one sample for that one lab.

So for the remediation of the errors, the most common error we saw in set one was confirmation false negative due to interference from high levels of codeine and morphine. One laboratory that had interference from codeine and morphine also had quantitation problems with oxycodone. That lab switched from GC-MS to LC-MS/MS and retested all of the samples in set one. Second laboratory validated an alternate GC-MS method to use when interference was observed in a primary method. Then other labs revised their methods to be able to resolve the interference.

The only errors that we saw on opioid PT samples in set two were minor quantitation errors. So those that were more than 20 percent from the mean but less than 50

percent. Laboratories are not required to submit a remedial response for this type of error.

In set three, we had two confirmation false negatives for oxycodone in a sample containing noroxycodone and noroxymorphone. At one laboratory, the wrong peak was integrated, causing failing ion ratios. This lab proposed staff retraining as their corrective action. At the second laboratory, the sample was analyzed by both a primary and an alternate method, but ion ratios failed in both analyses. This lab proposed modifications to either their GC-MS method or their extraction procedure.

There were 11 minor quantitative errors in set three. Nine of the 11 errors were made by a single lab for oxycodone and oxymorphone. This lab had prepared their calibrator and controls in-house in April and used them for all three qualifying sets. So as part of their investigation into their errors in set three, they purchased a calibrator from a commercial source and discovered that the oxycodone and oxymorphone in their in-house calibrator had degraded. The lab indicated that they would purchase a calibrator from the commercial source in the future. Then kind of as a side note, this lab indicated that they were also interested in switching from GC-MS to LC-MS/MS.

Then for the one major quantitation error we had for hydrocodone due to interference from norhydrocodone, the lab developed an alternate method that was able to resolve the interference.

So in summary, there were no false negative immunoassay results for samples containing the new opioids at 1.25 times the initial test cutoff. Confirmatory quantitative challenges had minor error rates similar to what we see for codeine and morphine. All required remedial actions for the PT errors will be completed by October 1. The data for the qualifying PT samples and the validations of the methods used to test those samples will be reviewed at NLCP inspections starting this week, actually. Overall, we feel like the labs are prepared for implementation on October 1.

Questions?

MR. MAKELA: Thank you, Cynthia. Any questions from board members? All right, no questions. We're quite a bit ahead. So why don't we take a short five or ten-minute break? Our next presenters are going to be presenting remotely, just make sure they're all set up to make their presentations from a remote location. So we'll reconvene at about 10:25 and then we'll resume with the agenda from there.

(Brief recess.)

MR. MAKELA: So next up, we're going to have Dale Hart, from RTI International. He's going to present about the pilot program for oral fluid performance testing. So I'll turn it over to Dale, and one recommendation, talk really slow, so we don't have another unexpected -- we have a break after your presentation anyway, so go ahead, Dale.

Agenda Item: Oral Fluid Pilot PT Program: Lessons Learned, E. Dale Hart, Research Forensic Scientist, Center for Forensic Sciences, RTI International

MR. HART: All right, thanks, Brian. Good morning, I'm Dale Hart from RTI International. I work with the national laboratory certification program. To give you a little bit of a background about myself, I've been at RTI since 1995, so about 22 years. First three of those years were as a contractor, and the last 19 has been as a regular employee, and most of the time here I've worked with the PT programs; that is, I'm one of the chemists that actually makes the samples.

And today I'll be talking about the NLCP oral fluid pilot PT program. I'll be discussing lessons learned in our most recent round of the program.

The oral fluid pilot PT was initiated in April 2000, with 21 occasions, or rounds, being conducted through 2007. Then the program was suspended for four years before

being restarted in 2011. Since 2011 we have conducted an additional 24 occasions.

Currently the program is voluntary and open to any laboratory that is willing to submit a letter of commitment and information on test methods. The most recent round was occasion 45. The survey is not graded, and there is no remediation.

Here is the overall process for the pilot PT. We develop a sample scheme based on NLCP needs, formulate the samples 1-2 days prior to shipment and freeze them. And ship the samples on cold packs. Laboratories are provided with a preformatted Excel spreadsheet for reporting their results, and are to return their results within two weeks of the receipt of the samples. After receiving all laboratory results we reduce the data and present the results to the participants as a webinar. Finally, we transmit aggregated results tables to the participants.

Today I will be discussing the most recent round, occasion 45. At this point, the data that we received from the laboratories are very consistent, occasion to occasion. Therefore we can make some assessments about overall laboratory performance using this occasion as an example.

In this occasion we were interested in evaluating three things in reviewing the data from the laboratories. One was the performance of the participating laboratories

in analyzing samples at low concentrations. Second was the effect of interfering compounds on the results obtained by the laboratories. And finally, the results of samples prepared in synthetic oral fluids versus results for paired samples prepared in human oral fluids.

Occasion 45 was shipped to the laboratories on February 15 of this year. First, I will start with the composition of the laboratories in occasion 45, which is shown on this slide. We had one laboratory that was initial testing only, and one laboratory that was confirmatory testing only. All other participants had both initial and confirmatory testing capability. Reagent manufacturing companies were represented by three laboratories.

The basic scheme for the first eight samples for occasion 45 is shown on this slide. Sample 1 contained THC at 40 nanograms per milliliter, and carboxy-THC at 50 picograms per milliliter. Sample 2 contained cocaine and benzoylecgonine at 3.2 nanograms per milliliter each, and PCP at 4 nanograms per milliliter.

Sample 3 contained racemic amphetamine and methamphetamine at 20 nanograms per milliliter each. These samples were prepared to evaluate the laboratory's capabilities at testing for amphetamines' stereoisomers. Please note that for sample 3, each individual

methamphetamine and amphetamine stereoisomer is really at 10 nanograms per milliliter.

Sample 4 contained MDMA and MDA at 25 nanograms per milliliter each, and oxymorphone at 15 nanograms per milliliter in human oral fluid. Note that sample 5 is the companion sample to sample 7, which contained the same analytes in synthetic oral fluid.

Sample 5 contained 6-acetylmorphine at 0.8 nanograms per milliliter in the presence of a high concentration of morphine as a potential interfering compound. Sample 6 contained cocaine and benzoylecgonine at 8 nanograms per milliliter each, and PCP at 10 nanograms per milliliter. Again, sample 7 was the companion to sample 4.

And sample 8 contained oxycodone and oxymorphone at 6 nanograms per milliliter each in the presence of a high concentration of morphine as a potential interfering compound.

Continuing, sample 9 contained codeine and morphine at 6 nanograms per milliliter each, in the presence of a high concentration of oxycodone as a potential interfering compound. Sample 10 contained hydrocodone and hydromorphone at 6 nanograms per milliliter each, in the presence of a high concentration of morphine.

Sample 11 contained codeine, morphine, and hydromorphone at 6 nanograms per milliliter each, in the presence of hydrocodone, noroxycodone, and norcodeine as potential interfering compounds. Sample 12 contained THC at 60 nanograms per milliliter, and carboxy-THC at 75 picograms per milliliter as a glucuronide.

Sample 13 contained THC at 0.8 nanograms per milliliter, and it was a blank for carboxy-THC. That is, the labs were directed to test for carboxy-THC in that sample. Sample 14 is the companion sample to sample 3, which was on the previous page, and contains racemic amphetamine and methamphetamine at 20 nanograms per milliliter, this time in human oral fluid. And finally, sample 15 contained 6-acetylmorphine at 2 nanograms per milliliter in the presence of hydrocodone and hydromorphone at 45 nanograms per mL each as potential interfering compounds.

Please note on these slides that the CT under the challenge column indicates that these samples were for confirmatory challenges for the included analytes. Initial testing was not required for occasion 45, and confirmatory testing was directed by analyte for each sample.

This slide shows the distribution of confirmatory testing for occasion 45. The majority of laboratories used LC-MS/MS. GC-MS was used only for PCP by two laboratories

that used LC-MS/MS as their primary confirmatory method. And GC-MS/MS was used only for carboxy-THC by a laboratory that primarily used LC-MS/MS for all other confirmations. Please note that the total number of laboratories expected to report confirmatory test results is 14.

The summary of the results for eight analytes -- THC, carboxy-THC, cocaine, benzoylecgonine, methamphetamine, amphetamine, MDMA, and MDA -- are shown on this slide. I will go over the relevant highlights for the results. Please note that there are ten columns on the slide: sample ID, analyte, target concentration in nanograms per mL, mean in nanograms per mL, standard deviation, N or the number of results included in the calculations, percent CV, the range of reported values, the number of outliers, and comments. Outliers are defined as values with a deviation from the mean of more than 50 percent.

I have highlighted samples 3 and 14 for methamphetamine and amphetamine, since they are companion samples. Sample 3 was prepared in synthetic oral fluid and sample 14 was prepared in human oral fluid. Also, samples 4 and 7 were companion samples for MDMA and MDA. I have highlighted the percent CV in red for any sample where the percent CV was greater than 17 percent. I used 17 percent as the cutoff because we use that criterion in the NLCP

urine PT to indicate unacceptable variability. In the urine program we do not grade such samples.

Overall the results from these eight analytes were good. In most cases, 13 of the 14 participating laboratories reported results for the samples. Only sample 6, for benzoylecgonine at 8 nanograms per milliliter, had results from all 14 laboratories.

The lowest number of reported values was two, for carboxy-THC samples; only two laboratories performed that test. The second-lowest number of reported values was 10, for THC in sample 13. In the N column it shows 9, but there's also an outlier, so the outlier was not included in the N column.

There were only two cases for which the percent CV exceeded 17 percent. That was cocaine in sample 2, with a target value of 3.2 nanograms per mL, and MDMA in human oral fluid in sample 4, with a target of 25 nanograms per mL. And there was only one outlier observed for these analytes.

On the next few slides I'll detail the results for carboxy-THC, which need some comment, and the results for the companion sample sets.

Analysis for the metabolite carboxy-THC was requested for samples 1, 12, and 13. All three matrices were synthetic oral fluid. In sample 12, the carboxy-THC

was present only as the glucuronide conjugate, in a concentration equal to 75 picograms per mL of free carboxy-THC. Sample 13 was a blank. Carboxy-THC results were similar lab-to-lab for sample 1, with lab B being than lab A. Note that the target concentration for this sample was 500 picograms. It turned out that the mean was 500, but note that one lab had a low result and the other had a high result.

However, when glucuronide of carboxy-THC was added, only lab B produced a result consistent with the total carboxy-THC, which was targeted at 75 picograms per mL of free THCA, indicating partial hydrolysis of the conjugate with lab A.

And these are laboratory-detected carboxy-THC in sample 13, as expected.

The total methamphetamine data from samples 3 and 14 are shown on this slide. As noted previously, these two samples are companion samples, and sample 3 was in synthetic oral fluid while sample 14 was in human oral fluid.

Overall agreement with theoretical was good; five slightly high. CVs range from 10.7 for sample 3 to 11.6 for sample 14. All submitted data from both samples were usable to calculate the mean, that is, there were no outliers. The reason for the difference in reporting

laboratories -- 13 laboratories for sample 3 and 12 laboratories for sample 14 -- is that 20 nanograms per milliliter is the LOD for one of the participating laboratories. One of their results was less than 20 nanograms per milliliter, and was not reported. The slight differences in the results for these two samples were statistically significant.

The total amphetamine data for samples 3 and 14 are shown on this slide. As was true for methamphetamine the two samples were companion samples for amphetamine. Here agreement with theoretical was overall good. CVs ranged from 15.7 percent for sample 3 to 14.7 for sample 14. And again, as you can see by the overlap of these, there's no difference in the results. The results were not statistically significant.

The data for stereoisomeric percentages of methamphetamine and amphetamine in samples 3 and 14 are shown on this slide. Remember, again, that these samples are racemic mixtures of both methamphetamine and amphetamine, so we should expect to see about 50 percent for the percent D and percent L.

Agreement with theoretical was overall good for all methodologies. However, only three participants demonstrated the ability to determine stereoisomeric percentages at 10 nanograms per mL for each stereoisomer in

human or synthetic oral fluid. That is, only these three laboratories report D and L isomers.

The data for MDMA from sample 4, which was in human oral fluid, and sample 7, which was in synthetic oral fluid, are shown on this slide. There were no outliers in either data set. Agreement with theoretical was good with a slight negative bias. The CV for the human oral fluid sample was 17.7 percent, while the CV for the synthetic oral fluid was 13.7 percent. Although that CV for the synthetic fluid sample was lower, the difference is not statistically significant.

The data for MDA from samples 4 and 7 are presented on this slide. As was true for MDMA, the two samples containing MDA are companion samples. Sample 4 in human oral fluid, sample 7 in synthetic oral fluid. Agreement with theoretical was overall excellent. The CVs range from 13.9 percent for sample 4, to 14.5 for sample 7.

And as was true for all of the other companion samples, the samples do not appear to demonstrate any significant difference with respect to recovery and inter-laboratory variation between a synthetic oral fluid sample and a human oral fluid sample.

The summary of the results for the remaining eight analytes -- codeine, morphine, 6-acetylmorphine, hydrocodone, hydromorphone, oxycodone, oxymorphone, and PCP

-- are shown on this slide. I will go over relevant highlights for the results. Again, I've highlighted sample IDs for samples 4 and 7, which are companion samples for oxymorphone.

As with the first eight analytes, most of the laboratories reported results for most of the samples. The lowest number of reported values were for 6-acetylmorphine at 0.8 nanograms per mL, which is sample 5, with eight reported; that is, seven that were in calculations, and one outlier. And oxymorphone at 6 nanograms per mL, sample 8, with nine reported values.

As can be seen from the comments column here, most of these samples were prepared with analytes in the presence of potential interfering compounds. A likely consequence of this is that the higher number of samples for which the percent CV was greater than 17 percent. There are five cases here. In addition, there were six outliers for these eight analytes. I should note, however, that excluding carboxy-THC, there were 37 analyte challenges in this set of PTs, and of the seven, there were seven cases for which the percent CV was greater than 17 percent. There were also seven outliers across the 37 analyte challenges, but those analytes were produced by only three laboratories. Two laboratories produced three

outliers each, and one laboratory produced one outlier. So 11 of the participating laboratories produced no outliers.

Here I'd like to highlight the oxymorphone results for the companion samples 4 and 7, with oxymorphone added to each sample at a level of 15 nanograms per milliliter. Sample 4 was in human oral fluid; sample 4 had one outlier, while there were no outliers in sample 7. Overall agreement with theoretical for the samples was excellent. The CV for sample 4 was 14.1 percent, while the CV for sample 7 was 13.8 percent.

Please note that the laboratory that produced the outlier for sample 4, which was at 34 nanograms per milliliter, and indicated by the red dot, reported 15 nanograms per milliliter for sample 7.

So here are some observations about the laboratory performance based on the data from occasion 45. As I noted before, the results seen in this occasion are indicative of our observations for the last couple of years. That is, round-to-round, we see very similar results, regardless of the drug we send and their concentrations; we get generally about the same performance.

First, laboratory performance was good overall, with most laboratories reporting results for most of the samples. This included at low concentrations. In total,

across all samples, excluding carboxy-THC, there were 457 results reported in this PT occasion, and there was a total of only seven outliers.

Second, interfering compounds did appear to have some effects for data in this occasion. In the cases of opiates and opioids at low concentrations, we observed nominal increases in CVs when samples included potential interfering compounds, and an increase in the number of outliers.

Third, results for paired human and synthetic oral fluid samples were statistically the same. Therefore, we can prepare PT samples in either matrix. We observe in other work that samples prepared in synthetic oral fluid samples do exhibit better long-term stability, primarily for cannabinoids and 6-acetylmorphine. So the use of synthetic oral fluid may be preferred if samples were to be prepared in advance and stored.

And overall, as we've seen for really the last couple of years, in conclusion, we have a group of about seven to eight labs that are capable of meeting the analytical requirements that would be required in the 2015 proposed guidelines. And as I say, this observation's been pretty consistent for the last couple of years.

And with that I'll take any questions.

MR. MAKELA: Any questions from board members?

DR. SCHAFFER: Hi, Dale. Mike Schaffer. I was told that the survey was on hold for a while. Why is that?

MR. HART: We were just directed to put it on hold for a bit.

MR. FLEGEL: This is Ron. I am going to clarify, since that question was asked. It actually wasn't asked necessarily to be put on hold, but to conserve funds we actually looked at the program as an overall, because there were so many things going on with the implementation of the urine. So we had to look that, overall, programmatically. So, again, I was going to say a couple of things, and I'll say them in a little bit.

MR. MAKELA: Thanks, Mike. Any other questions from board members? Anyone? Okay, before we go on break, Ron had a few more words.

MR. FLEGEL: Thanks, Dale. I just wanted to say to the board members as an overall, with the board looking at the program, specifically around the PTs, the last two. I wanted to thank both the labs, specifically, and NLCP. When we make these up, these are very complex, in what we're looking at -- the laboratories, in performing the testing and everything around that. So it is complex when we come up into a proficiency testing program for the labs, and it is a lot of work, obviously, overall, for both NLCP as well as the labs.

But I think, as you can see, under the program, I think we are ready to implement October 1. I think the synthetic opioids, which are in combination a lot of times, we'll see, in a lot of the testing we see there's combination drugs, that I think, overall, with interference we've concluded that the laboratories can perform the testing and not have interfering substance, whether it's an interfering substance or another synthetic opioid.

I think the same with the oral fluid. We're getting to the point where I think we're pretty confident in the results from the laboratories. I think we now have to start challenging the laboratories with the initial testing phase for oral fluid, especially since most of the laboratories have performed this testing overall for drug analytes other than the semisynthetic opioids, so I think that will be something that we'll have to look at closely.

And I did want to clarify just a couple things that were pointed out. One was, I think I had said June 1, 2018, for the extension of the CCF. That should actually be June 30. It may have said that on the slide. But again, the question came, would it be extended again? And again, we would look at that; if it had to be extended further, we would extend it further, through OMB approval.

And again, one other mention that I should have mentioned is, within the MRO guidance manual, again, we're

not necessarily looking for comments for that, but if there are any comments that an MRO would see that could come back to us, we would welcome that. But the other part of that is, which I think is going to be very informative going forward, is we're also working on case studies. And those case studies, once we've concluded those, and finished them, we hope to post those also like we've done with other urine case studies around the opiates, morphine and codeine.

So I think those are important, as far as case studies. And again, if there's MROs that have flowcharts that they use, I would definitely welcome to see those. I think those are very important, of how people look at those. Dr. Ed Cone had a great presentation on the opioids, and I think there's so many combinations of what you can see and the answer you should give as an MRO.

So again, if there are any flow charts that individuals use or MROs use or labs use, when certifying scientists, we would welcome those, too. Thank you.

MR. MAKELA: Thanks, Ron. With no other comments, we'll go on our scheduled break. It goes until 11:15. So we will reconvene at 11:15 to continue the agenda. Thank you.

(Brief recess.)

MR. MAKELA: Welcome back, everybody. Continuing with today's open session agenda, next up is Dr. Robert White from RTI International. He is going to talk about pre-study about DNA in urine and oral fluid. So I will hand it over to Dr. White.

Agenda Item: Stability of DNA in Urine and Oral Fluid (A Pre-study), Robert M. White, Sr., Ph.D., DABCC, Senior Research Forensic Scientist, Center for Forensic Sciences, RTI International

DR. WHITE: Good morning. I'm Bob White. I'm retired from RTI International as of close of business 01 August, 2017. However, the work that I'm presenting, which involves the examination of DNA as an identity marker in urine or oral fluid collected for employment-related purposes was performed while I was an employee of RTI International. Today I am presenting only as the registered agent for the firm RMW Consulting Incorporated.

DNA, which stands for deoxyribonucleic acid, has grown from an almost unknown abbreviation in the 1980s to a current household word. Even though DNA is incredibly complex, it can be condensed to two strands or chains that interact with each other. Each strand or chain is composed of four bases, adenine, thymine, guanine, or cytosine, that are chemically bonded to the sugar deoxyribose. The deoxyribose molecules are linked by phosphate bonds. DNA

is found in the nucleated cells in the chromosomes and this mitochondrial DNA in the cytosol of cells.

Mitochondrial DNA does have forensic applications, but it will not be discussed in this very brief presentation. Nuclear DNA is distributed unequally throughout the 22 autosomes or non-sex chromosomes, and either two X chromosomes for human females or an X and a Y chromosome for male humans.

DNA is the biological blueprint for proteins which either are our structural building blocks or the enzymes that produce such building blocks for all human life. Human nuclear DNA is double-stranded. About 99.7 to 99.9 percent of human DNA is identical from human to human, making most human DNA useless for purposes of differentiating one human from another or for identity purposes.

We are going to look at the DNA that is useful for either differentiating or confirming identity. Structure 4 is a cartoon of the two strands of human DNA taken from the reference on the slide, the abbreviated structures of the four previously mentioned bases are presented on the slide, the adenine, thymine, guanine, and cytosine.

As noted on the slide, adenine usually binds with thymine and guanine usually binds with cytosine. Because

the GC bond contains three hydrogen bonds and the AT bond has only two, the GC bond is usually stronger and harder to break than the AT bond.

We won't be discussing the transcription of DNA into RNA and the translation of the RNA by triplet codon in the protein. However, the essential process is presented on the slide, as this is the basic function of DNA. RNA looks like DNA, except that the sugar is ribose and uracil replaces thymine. It is worth mentioning that within a gene, human DNA has protein coding regions called exons. Within the same gene, there are usually noncoding introns which have to be removed from the RNA that was produced by transcription of the DNA before translation, and the intron exon differentiation will become important later.

There also exist long stretches of DNA that probably do not contain a gene. In most of genetics, the coding regions of DNA are what is of primary interest. However, for forensic DNA, the opposite can be true, as will be pointed out today.

There are differences in some coding regions which may produce an aberrant protein or be silent and produce the same protein as the wildtype or most common gene. Such differences are called SNPs or single nucleotide polymorphisms. SNP is usually pronounced snip. A common example of a SNP is a replacement of an adenine

for a uracil in the RNA that produces the beta protein chain of hemoglobin. The nucleotide chain results in hemoglobin S, which has a valine, an amino acid, rather than a glutamine, another amino acid, at position 6 in the beta chain.

Fortunately SNPs are fairly rare and do not routinely make good candidates for differentiating one human from another. Another locus, which is just a Latin word for place, or place in which DNA is highly differentiated, is the human leucocyte antigen DQ1, sometimes called DQ-alpha region. The HLA system is what makes a human unique. The HLA-DQ1 system, plus a polymorphic marker, or PM system, have been used successfully to individualize urines for postmortem testing.

Another area on a given strand of DNA where one human many times is different from another is short tandem repeats, which are found on numerous different chromosomes. The STRs are up to 13 tandem repeats in number. They are sequences of DNA that occur in noncoding regions in different numbers and without any intervening DNA.

STRs currently are what is employed in much of forensic DNA identity testing. In most cases, STRs have varying numbers of repeats from one human to another, which

commonly is also known as length polymorphism, polymorphism just being a long word for many faces, from the Greek.

By determining a sufficient number of multiple STRs for a given human being, that human becomes unique with respect to their STRs. Given the analysis of enough STRs from different loci or places on different chromosomes and their known variability, an individual can be differentiated from another person or proven to be the same individual to a high degree of probability. When both copies of a DNA strand have the same number of a given repeat at a given place or locus, the individual is said to be homozygous. When the two copies of a DNA strand have different numbers of a given repeat, the individual is said to be heterozygous. Their actual purpose in life or the purpose of the STRs is debatable.

Examples of STRs commonly employed in human identification are shown on the slide. There are four columns in the table on the slide. The first and third columns are the locus or place where the repeat is found. The second and fourth columns are the repeated sequence. The markers and associated repeats on the slide look like what is commonly referred to as alphabet soup. However, the system can be categorized fairly easily, and that's what I would like to do for today is just break it down into something easy.

The first DNA STR marker in the upper left-hand corner of the table on the slide is D3S1358. That translates to D for DNA, 3 for chromosome 3, S for single copy, and the 1358th sequence. D3S1358 is not a part of any currently identifiable gene. In other words, it doesn't produce protein.

The next STR marker on the slide is TH01, not T-H-O-one, T-H-zero-one. TH is for the gene that encodes tyrosine hydroxylase, a common enzyme. The 01 indicates that the STR is in the first intron, which again is a nonprotein coding region.

The next two STR markers are designated similarly to D3S1358. However, they are on different chromosomes from each other and different from chromosome 3.

Penta-E on the long arm of chromosome 15 was added by Promega. Its counterpart, Penta-D, which is on the long arm of chromosome 21, is presented in the same marker column on the slide, and also was added by Promega.

The next four markers after Penta-E are designated similarly to D3S1358. The next marker, which is over in the second column, CSF1PO, is part of a gene that encodes for the protein C-FMS-1 protooncogene. The STRs from the sixth intron, which again is nonprotein coding, are used for CSF1PO.

As stated previously, Penta-D in the second column was introduced by Promega and is on a separate chromosome from Penta-E.

VWA or Von Willebrand's factor, is a very large gene. The STRs from the 40th intron or in a nonprotein region are used. The last noncoding region to contain an STR is D8S1179, which is designated similarly using the system previously described for D3S1358.

The last two STRs are TPOX and FGA, which are in the intronic regions of thyroid peroxidase. That's the TPOX, and fibrinogen A, or FGA, genes respectively. TPOX uses the 10th intron from the TPOX gene, while the third intron of the human alpha fibrinogen alpha gene is used for fibrinogen A is used.

Amelogenin is not an STR. Rather, it is a gene that codes for protein in tooth enamel, and due to differences between males, you can readily distinguish between a female and a male using amelogenin.

In our proposed study, we wanted to determine the approximate of degradation DNA might experience under routine storage conditions typical of a drug testing laboratory. The study was RTI, IRB-approved. We had five donors, two females, three males. All samples were collected, self-collected, simply because these are all people who are experienced in collecting both urine and

oral fluid. We collected an A and a B urine and oral fluid. So both urine and oral fluid had an A and a B bottle collected on them. No drug testing was included in the study.

The samples were stored on the RTI campus in an air-conditioned office prior to transportation to the testing lab. They were stored there in my office for a couple of days. The samples were transported to the testing laboratory, which is LabCorp Genetica over in Burlington, North Carolina, by a courier car, which turned out to be my car. FedEx, UPS, and commercial air were not a part of the transportation system. So we have no idea from this pilot study what the effects of being up at 35,000 feet may or may not be.

Urine. It contains some nuclear DNA, probably mostly due to the presence of intact epithelial cells from the bladder, kidney, and ureters. Urine is hypertonic. It is not a favorable environment for DNA or intact cells. For urine, we are going to analyze the STRs at 5 days post-collection, because that's the amount of time it took to get from here in my office after collection, wait over a weekend, and then over on a Monday is when these samples were actually accessioned and analyzed.

And 35 days post-collection in a B bottle. The B bottles were frozen upon accessioning.

There's just a standard look at a couple of urine bottles. It should be very common to anybody who does urine drug testing, and there's an associated chain of custody, but this was not included with the pilot study, did not accompany the samples.

Oral fluid. Well, oral fluid originates from the same cavity in which buccal swabs for DNA are obtained. Buccal swabs are a standard LabCorp Genetica collection, and that's also where the oral fluid comes from. Neat oral fluid and buccal swabs were analyzed at five days, which again is a couple of days for transportation and over a weekend and accessioning on the following Monday, and 35 days post-collection. Pad-type devices were analyzed at 5 days post-collection and 39 days post-collection, simply because that's the way the lab operates.

The neat oral fluid was expectorated into a tube, and the pad-type devices are the device that I think everybody will become very familiar with where you put a pad in your mouth, leave it until there's an indicator that says you have enough oral fluid, and then put it into a preservative buffer solution and then seal it up and send it off to the laboratory.

This is just on slide 13, a photograph of the four buccal swabs that went off to the lab and their envelope. Two out of the four were used by the laboratory

and two were there for a backup. Four is just what the lab standardly collects. So we collected four.

Slide 14 is about 10 mL of neat oral fluid. We didn't collect this much. This is collected in a larger tube than we use. But what I want to point out is look at the amount of debris and cells that just settle in the bottom of just regular neat oral fluid. What is in the bottom of this tube is due to sedimentation. It is not due to centrifugation. This was not a centrifuged tube.

But what is in the bottom is very rich in nucleated cells. This is just a microdroplet from the bottom of the tube, the previous tube, stained with methylene blue, cover-slipped, and it's a 400x magnification. You can see numerous nucleated cells, along with some unidentified sediment, and the clumped cells most likely are bacteria that have been aggregated by IgA, which there is a lot of IgA in your mouth for that purpose, and I'm not even going to try to identify the cells. I'm not a pathologist. But you can probably pick out regular epithelial cells and some stellate cells.

We use the Biophore collectors for the study. I just didn't have a photograph of it. This is the tubes that are exactly the same size as the Biophore tubes with a Teflon-lined cap, and there's a quarter in there just to give you a good idea for comparison. We collected a

minimum of 2 mL of neat oral fluid in both the A and the B tubes.

For the pad-type collection, we use three commercially available pad-type oral fluid collectors. That is to say, again, the collector uses a fiber pad to collect the oral fluid from the donor's oral cavity, which is then placed into a buffer preservative and then employed in the study. The pads or the pad-type collectors were designated A, B, and C, and this should not be confused with the collection of an A and B tube for each type of collector.

For DNA extraction, the QIAMP DNA investigator system was used by LabCorp Genetica over in Burlington; for the quantitation, the QuantiFluor 1 double-stranded or DS-DNA system was used; and for the actual capillary electrophoresis where we are going to separate out those different bases, the 3130xl Genetic Analyzer was used to obtain 15 different short tandem repeats and amelogenin. That's the gender identifier. From the PowerPlex 16HS kit. Fifteen STRs, one amelogenin marker.

It's difficult to see each one of the individual STRs on this. It's a Foley electropherogram, as shown on the slide, that includes all the markers that we are going to look at. However, since it's too small to see as projected, because I know that's probably a pretty large

room up there, what I did is I cut off a corner that has only D3S1358, TH01, and D21S11 on it, and here you can see a plot of relative fluorescent units. This is peak height. That is what the DNA world operates off of, not peak area.

And then the x-axis is the base pairs compared to a known DNA ladder. It's a standard that is put in there. D3S1538 is a homozygote, since there's only major peak in there, and you see two red triangles. That is where the software looked for D3S1358.

You will also see, if there's any chromatographers out there in the audience, a small peak to the left of the major peak. This is what's called stutter. It's due to a strand slip during the polymerase chain reaction, which magnifies or amplifies the amount of DNA present. The software is programmed so that it ignores stutter, and the TH01 is a heterozygote with a 9.3 designating a variant. That 9.3 does not mean 30 percent. It just means that it was a truncated repeat or there were three more found than would be expected. This is not an unusual finding. It has nothing to do with saying the results of this comparison are either good or bad. Variants are out there and they are in the published literature.

Then D21S11 also a heterozygote. So there's two peaks in there, and then you see those two little stutter

peaks next to each one, and this is 29 33.2, again there's a .2 variant, and that's two more base pairs than expected, or you can look at it the opposite way. It was one of those base pairs had -- or one of those tandem repeats had two base pairs knocked off of it.

The results for each type of collector are shown on the slide, and the results are plotted as peak height. Again, that's how the DNA world operates, not off of area. On the y-axis for each type of device for day 5, which was 5 days post-collection, and either day 35 for urine, which was frozen, or neat oral fluid which was frozen, or day 39 for the pad-type collectors which were not frozen.

Each type of collector, please note, each type of collector shows relatively less fluorescent units at day 35 or 39 versus day 5, and that even includes the buccal swabs, which are the laboratory standard.

But just take a minute to look across at each one of the collectors and you can see that, indeed, when you go about a month beyond day of collection you're getting less DNA no matter what your sample was collected in or how it was collected.

This slide is mean percent profile. You saw on that electropherogram that we have 15 STRs that we want to look at and amelogenin. Well, what happens if you don't have enough DNA and you have a dropout and you only end up

with 14? Well, then on the slide it would be counted as not giving a complete profile. The buccal swab, you notice, was pretty good, although it looks like there's one dropout on there. Urine had a number of dropouts. Didn't get complete profiles certainly always, and the A, B, and C devices, same thing, and neat oral fluid was somewhere between urine and the buccal swab.

Looking at the urine results in a different fashion, this comes from the JMP program, which is produced by SAS over in Cary, North Carolina. Again, we are going to look at peak height in relative fluorescent units versus molecular weight in base pairs, and most toxicologists aren't used to looking at something in terms of base pairs. A base pair is probably about 600 amu. So you can multiply 600 times 400 and see that by the time we get to the end of the graph, we're dealing with about molecular mass of around 30,000. Pretty big molecule, but you'll notice that for the blue line, which is five days, and then going to the orange line, there is an obvious loss quantitatively after 30 days and one freeze/thaw cycle.

The usual pattern that we would see for buccal swabs, and maybe I should have -- and some of the collectors was more DNA or a hump down there around 160 to 240. These two curves are pretty flat.

A lot of comparisons were done in the JMP program. The buccal swab I've pretty much gone over; there was no real difference between day 5 and 35. A little more difference but not above .05, certainly for urine. For the neat oral fluid it looked about the same as the urine. For DNA loss on the buffer and pad, the curves looked pretty much the same, even though there was some loss, and you'll see that the P-value is less than .0001 in both cases. The buffer, when you compare it to the pad, on day 5 and day 39, how much was lost, you can see that everything stays above or below, excuse me, .05.

The B device was about the same for the A device, but the type of DNA loss was different. So you notice down here day 5 versus day 39, .1870, yeah, they were just not different. And the C device, it did the best as far as quantitatively holding the DNA or preserving it, but when you looked at the buffer versus the pad, there was a difference. One of the things I forgot to mention, that LabCorp Genetica was kind enough to do a special elution procedure for these oral fluid collector pads.

Conclusions. The A bottles were acceptable 5 to 6 calendar days post-collection. You got a complete profile and enough DNA to use. However, a complete profile was not obtained in 40 percent of cases after freezing B bottles until day 35 post-collection. Neat oral fluid did

about the same. You can compare this to the top bullet point by saying four out of five or 20 percent of the cases we didn't get a complete profile.

The oral fluid pad eluent and buffer preservative produced a complete profile for all collectors for all donors 5 days post-collection. When Erin called me up, she said you won't believe this. Everything produced a complete profile. That was on the initial round.

However, at 39 days post-collection, only the C pad and buffer preservative produced a complete profile after 35 days of storage, and the pilot study provides no stability data beyond 35 days for frozen urine or oral fluid, and the pilot study provides no stability data beyond 39 days for the pad-type collectors. Whether usable DNA can be extracted from a pad or its buffer preservative after extended storage needs to be the subject of a study that employs a longer storage time. This only went out to the maximum of 39 days for the pad-type collectors.

The project team involved three groups of people from three different organizations, RTI, and then you see the folks from LabCorp Genetica, Dr. Maha, Megan MacKenzie, Erin Hall, Kelly Rogers, and then the coordination from the folks at SAMHSA. So this was three organizations putting this pilot study together.

My thanks to everyone for their cooperation.

And that brings me to the end of my talk, which went maybe a little bit longer than I anticipated, but I think I'm still under my 30 minutes. So that's the end, and if there's any questions or comments, I'll be glad to try to handle them. Thank you.

MR. MAKELA: Thanks, Bob. Any questions from board members? No? I'd like to give an extra special thanks to Bob. He had some troubles with the weather events down in Florida, and he was still able to make the presentation today. So thanks, again, Bob.

DR. WHITE: You are most welcome. You just haven't seen anything until you've seen the eye of a Cat 4 go over you. But the house held together.

MR. MAKELA: Good to hear.

Okay, so moving on, next presentation we have Megan Grabenauer from RTI. She's going to talk about detection of opioid glucuronides in hair. So I'll wait a second while her presentation loads, and Megan, are you on the line? Once this all loads up, I'll just go back to the start for you, and you should have control. So go ahead and start it.

Agenda Item: Detection of Opioid Glucuronides (metabolites) in User Hair, Megan Grabenauer, Ph.D., Research Chemist, Center for Forensic Sciences, RTI International

DR. GRABENAUER: All right, thank you. So as Brian mentioned, my name is Megan Grabenauer. I am a research chemist at RTI International.

In this presentation, I will go over some work that we have done in our labs at RTI looking into the presence of opioid glucuronides in drug user hair. So the purpose of this work is to add to the body of scientific knowledge related to drug testing in hair in support of the proposed revision to the mandatory guidelines to allow the use of hair, sweat, and oral fluid, as alternative matrices to urine.

Hair is a desirable matrix for drug testing for a number of reasons, one being because it's less invasive to collect than urine, it's easier to conduct a controlled or observed collection, since there are fewer privacy concerns. Hair is also more difficult to substitute or adulterate, and it provides a longer window of detection. One tradeoff for this longer window of detection, however, is that it does now show recent use since it does take time for the hair to grow out.

Currently, the use of hair as a matrix for drug testing is limited by issues that may affect the defensibility of the testing result. One of those issues that is unresolved to date is the issue of possible external contamination. So external contamination refers

to the situation where drug is incorporated into hair from a mechanism other than drug use. It might happen as a result of secondhand passive exposure while in proximity to others who are using drugs. It may happen in the course of job related duties for those who work around controlled substances, or possibly coming into contact with contaminated surfaces such as in a hotel.

Currently, most drug testing in hair targets parent drugs, which can be transferred to hair via external contamination. So in order for hair to be a reliable testing matrix, there needs to be confidence that a positive result is indicative of drug use and not there from external contamination.

There have been a number of research efforts to date to address this issue. One approach involves using an extensive wash procedure and analyzing the final wash as an estimation of how much external contamination may be retained after the wash procedure. The theory here being that external contamination is not incorporated into the hair in the same manner as drugs deposited through metabolic processes. So external contaminants can be washed away while incorporated drug metabolites are retained.

Another approach has been to use metabolites instead of the parent drugs as targets. One thing to

consider here, though, is that many drug metabolites are present as manufacturing impurities or degradation products. So efforts in this area have recently included establishing expected ratios for drug use that are distinct from ratios one would find as a result of contamination, and the approach that I'll focus on today is identifying unique metabolites of use that cannot be a result of external contamination.

So a quick review for those of you who may not be familiar. Metabolism is the chemical process by which the body changes drugs into forms that can be eliminated. It proceeds in two phases. Phase I metabolism generally unmasks or inserts a polar functional group, and the most common of these transformations are oxidation, reduction, and hydrolysis. Phase II metabolism is sometimes referred to as conjugation. The most common of these transformations are glucuronidation, acetylation, and sulfation.

The conversion of heroin to 6-acetylmorphine is an example of the hydrolysis reaction, and this hydrolysis can happen through phase I metabolic transformation or through an in vitro hydrolysis process, either taking place in solution or from moisture in the air. So this is an example of phase I metabolite which does not necessarily indicate use when it is found on hair.

Amphetamine is transformed into 4-hydroxyamphetamine via oxidation, and this oxidation can occur via metabolic enzymatic processes or in vitro from hair treatments, including bleaching using peroxides.

Oxycodone is transformed into oxymorphone via oxidated dealkylation, and this is an example where a phase I metabolite is another prescription drug and is therefore not an ideal marker of use of the parent compound.

One of the most common phase II metabolic transformations is conjugation with glucuronic acid to form a glucuronide conjugate, and this process is shown here for the formation of codeine-6-glucuronide. So phase II metabolites are ideal markers of use, because as opposed to phase I metabolites, they are not products of common degradation pathways, they're not manufactured impurities, such as in, say, benzoylecgonine, which you often find in street level cocaine. Phase II conjugated metabolites are not formed by in vitro reactions on the surface of hair, and they are not commercially available drugs.

This research was prompted by the need to identify metabolites for use in hair testing that are not present from external contamination. Very little progress has been made in this area for opioids in particular. So we did some preliminary studies looking for glucuronide conjugates of opioids in hair.

Those studies revealed that opioid glucuronide conjugates were present in the hair of known drug users. RTI then proceeded to develop and validated a quantitative method for several opioids and their glucuronides in the hair, and that method was used to analyze 46 user hair samples from our drug user hair inventory that had previously been confirmed positive for opiates.

So our method consists of washing the hair using the isopropanol and phosphate buffer wash, which has been published by Cairns et al. We used 25 milligrams of hair to which 500 microliters of extraction solvent is added. The hair is heated to 100 degrees C for one hour, cooled back down to room temperature, and then cleaned up with a solid phase extraction.

Note that in this method, the hair is not pulverized. The extraction solvent is M3 reagent from Comedical, and the process of the extraction does not digest the hair. The hair retains its form.

The manufacturer instructions for this extraction solvent state that the supernatant can be analyzed directly, but we found that without the SPE step, our chromatography was poor and our signal intensity was very low. So we needed to have that SPE in there.

Samples were analyzed by LC-MS/MS, using an Agilent triple quad and an Agilent LC system and

electrospray ionization. Our column was a C18, flow rate 500 microliters per minute, and our column temperature at 50 degrees C. The mobile phase was 5 millimolar ammonium formate with .1 percent formic acid as mobile phase A. Mobile phase B was methanol with .1 percent formic acid. The gradient goes from 5 percent organic up to 90 percent organic in 6.1 minutes, with a re-equilibration time on the end.

Retention times for all the analytes are shown here. So for all analytes except the morphine glucuronides, two transitions were monitored. For the morphine-3- and morphine-6-glucuronide, the abundance of the qualifier ion transitions that we found from the literature and are employed in assays for urine or serum, and even the ones that we found experimentally during analytical optimization of standards were unacceptably low in our extracted calibration curves, and we could not establish reliable ion ratios.

So in order to increase the sensitivity for these analytes and others that were in the same segment, the low intensity qualifier transitions were not included in the final method, and I would like to mention here that the selection of included analytes was limited by the availability of reference standards at the time.

We did use deuterated analytes as internal standards and monitored one transition for each.

Shown here are the LC-MS/MS quantifier transitions of the six glucuronide metabolites, which are each at 30 nanograms per mL, and eight opioids and 6-acetylmorphine, that are present at 450 nanograms per mL. The labeled transitions are the peaks that are filled in with green, and other analytes with the same transitions are shown in yellow. We did not see evidence for in-source fragmentation of the glucuronides, and we would notice that for example, by the presence of a peak in the morphine transition, showing up at the retention time for the morphine glucuronides. That would indicate that morphine glucuronide traveled the length of a column intact and then disassociated in the source to produce morphine in a quantification.

With this LC method, we were able to baseline resolve morphine-3-glucuronide and morphine-6-glucuronide from each other, as well as from the hydromorphone-3-glucuronide.

So these are validation results. You'll notice a dihydromorphine-3-glucuronide is not included there with an LOQ or ULOL. That's because it interfered with the morphine-3-glucuronide internal standard. So we decided to omit dihydromorphine-3-glucuronide from our standard mix in

favor of having reliable quantification of the morphine-3-glucuronide. But we kept the transitions in there to monitor for its presence.

Our calibration range was 2 to 120 picograms per milligram for glucuronides, except for the dihydrocodeine-6-glucuronide, which was only linear up to 80 picograms per milligram. The calibration range was 40 to 1200 picograms per milligram for the parent analytes. We also validated an equivalent to a dilution procedure in which we only used 10 milligrams of hair as opposed to the 25 milligrams in order to increase our upper limit of quantification to 3000 picograms per milligram.

So matrix effects aren't shown here, but they were apparent, as is expected for an LC-MS/MS method. However, they were controlled for by the internal standards.

The extraction recovery and overall process efficiency are lower than ideal. Further time could be spent optimizing the extraction method, and it would be worthwhile to provide an increase in sensitivity. I'd like to emphasize that this work is early stage data on the presence of opioid glucuronides in human hair. The analytical method was validated and some preliminary optimization was performed, but it was not fully optimized.

In addition to ruling out interferences from matrix and internal standards, six potentially interfering compounds were investigated to determine if they interfered with the analytes of interest. They were heroine, norcodeine, norhydrocodone, normorphine, noroxycodone, and noroxymorphone. Aside from the presence of heroin producing a signal for 6-acetylmorphine, no other compound produced interference in blank samples or caused any of the quantifications to be out of tolerance.

We also investigated the possibility of glucuronide conjugate production during the extraction process and did not see evidence for this happening in the presence of any of the analytes of interest. Interference with the same m over z as morphine-6-glucuronide was detected when morphine was spiked at 3000 picograms per milligram on the hair, but it could be separate from the morphine-6-glucuronide using alternate LC method. So we know that it was not actual morphine-6-glucuronide formation. No other analytes produced a similar interference, up to 3000 picograms per milligram.

So this method was then applied to 46 hair samples that had previously confirmed positive for opiates. The most commonly detected analytes were hydrocodone, morphine, oxycodone, and 6-acetylmorphine. So notably, our

sample set had no specimens with dihydrocodeine or dihydromorphine greater than 200 picograms per milligram.

As an external check on our quantitative accuracy, we compared RTI's quantified results with the confirmation results from Psychemedic's analyses of the same samples for analytes that were common to both methods.

There was very good agreement across the board for all analytes in all samples, and this was especially promising since many of these samples were sent to Psychemedics several months or even up to a year before they were extracted and tested at RTI. The samples were washed by RTI near the time of receipt, and Psychemedics did not perform any additional washing once they received the hair.

There are two discrepancies in the number of positive samples, one for hydrocodone and one for morphine, and in both of these instances, the quantified results were very close to the cutoff.

So 200 picograms per milligram has been put forth as a suggested cutoff for calling a sample positive based on parent drug concentration. What you see in the middle column are the glucuronide concentration ranges for the positive samples. The lowest glucuronide concentrations were 2.86 and 2.37 picograms per milligram respectively for codeine and hydromorphine positive samples.

Now, our lower limit of quantitation was 2 picograms per milligram, but we estimated concentrations to reveal glucuronides present at approximately 1 picogram per milligram for oxymorphone and morphine-positive samples. Codeine-6-glucuronide had the highest abundance relative to parent concentration, and morphine-6-glucuronide was present consistently higher concentrations than morphine-3-glucuronide.

This is a plot of morphine-3 and -6 glucuronide concentrations as a function of the parent morphine concentration. Here you can see that the morphine-6-glucuronide is consistently greater than morphine-3-glucuronide, and the general trend is the glucuronide concentration increasing as the parent concentration increases.

Twelve out of 16 samples that were positive for morphine had morphine-6-glucuronide concentrations above 2 picograms per milligram, and all 16 morphine-positive samples had morphine-6-glucuronide at 1 picogram per milligram or higher. Eleven out of 16 of the morphine-positives had morphine-3-glucuronide concentrations above 2 picograms per milligram, and 15 out of 16 morphine-positives had morphine-3-glucuronide at 1 picogram per milligram or greater.

These are the results for the codeine or the plot of codeine-6-glucuronide concentration as a function of codeine. All five samples that were positive for codeine had a codeine-6-glucuronide concentration above 2 picograms per milligram. There were two additional samples with codeine-6-glucuronide concentrations higher than 2 picograms per milligram and had codeine concentrations of only 118 and 78 picograms per milligram.

This is a plot of oxymorphone glucuronide versus oxymorphone concentration. Five out of six samples that were positive for oxymorphone contained oxymorphone-3-glucuronide greater than 2 picograms per milligram, and that sixth one had the glucuronide at greater than 1 picogram per milligram.

There was one additional sample with oxymorphone-3-glucuronide above 2 picograms per milligram that had an oxymorphone concentration of only 134 picograms per milligram. Since oxymorphone is a metabolite of oxycodone, the oxymorphone-3-glucuronide could have originated from oxymorphone or oxycodone present in the sample, and I won't get into the nitty gritty details here, but our dataset does suggest that its origin was from oxymorphone in the hair.

Two out of three samples that were positive for hydromorphone had hydromorphone-3-glucuronide

concentrations above 2 picograms per milligram. Hydromorphone-3-glucuronide present in the samples could have originated from hydromorphone or hydrocodone, and although our dataset is limited, it indicates that hydromorphone-3-glucuronide mostly originated from the hydromorphone.

In summary, codeine-6-glucuronide, oxymorphone-3-glucuronide, morphine-3-glucuronide, morphine-6-glucuronide, and hydromorphone-3-glucuronide are present in drug user hair and generally increase with parent concentrations. The glucuronide concentrations are approximately 1 picogram per milligram or greater and samples with more than 200 picograms per milligram of parent compound present. Now remember, our validated LLOQ was 2 picograms per milligram. So this is this estimate, and we highly suggest that future assays for glucuronides in hair should target an LLOQ of less than 1 picogram per milligram.

Using a cutoff of 200 picograms per milligram of parents and 1 picogram per milligram for glucuronides, 16 out of 16 morphine positive samples were positive for a morphine glucuronide. Five out of five codeine positive samples were positive for codeine-6-glucuronide. Two out of three hydromorphone-positive samples were positive for hydromorphone-3-glucuronide, and six out of six oxymorphone

positive samples were positive for oxymorphone-3-glucuronide.

The maximum relative abundance of glucuronides was less than 3 percent of parents for all but codeine-6-glucuronide, which was present at a slightly higher relative amount of up to 6 percent.

There's definitely room for improvement in our extraction method to increase the process efficiency and sensitivity, and unfortunately we did not have enough data from hair samples containing dihydrocodeine and dihydromorphine to draw conclusions about the presence or absence of their glucuronide metabolites.

So a manuscript with the full method details, and results has been submitted to the journal of analytical toxicology, and actually as of yesterday it has been fully accepted. So if you want the nitty gritty details, feel free to look up that publication.

And I'm happy to take any questions.

MR. MAKELA: Thank you, Dr. Grabenauer.

Any questions from board members about the information presented?

(No response.)

Nobody, huh. That's surprising. Okay, well, unless there were any further comments, Ron, did you have anything else to say? So since there were no registrations

for public comments, I'm just going to move to adjournment. So thank you, everybody, for attending today. I hereby adjourn this open session of the Drug Testing Advisory Board.

Again, if you want information on this meeting, it will be posted to the website and the earlier presentations in approximately three to four weeks. Thank you for your attendance. We look forward to the next meeting in December. Thank you, everybody.

(Whereupon, the open session was adjourned at 12:05 p.m.)